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Abstract:	Repression of the cellular CIITA gene is part of the immune evasion strategy of the γherpes virus Epstein-Barr virus (EBV) during its lytic replication cycle in B-cells. In part this is mediated through down regulation of MHC class II gene expression via the targeted repression of CIITA, the cellular master regulator of MHC class II gene expression. The repression is achieved through a reduction in CIITA promoter activity initiated by the EBV transcription and replication factor Zta (BZLF1, EB1, ZEBRA). Zta is the earliest gene expressed during the lytic replication cycle. Zta interacts with sequence specific elements in promoters, enhancers and the replication origin (ZREs) and also modulates gene expression through interaction with cellular transcription factors and co-activators. Here we explore the requirements for Zta-mediated repression of the CIITA promoter. We find that repression by Zta is specific for the CIITA promoter and can be achieved in the absence of other EBV genes. Surprisingly, we find that the dimerization region of Zta is not required to mediate repression. This contrasts with an obligate requirement of this region to correctly orientate the DNA contact regions of Zta to mediate activation of gene expression through ZREs. Additional support for the model that Zta represses the CIITA promoter without direct DNA binding comes from promoter mapping that shows that repression does not require the presence of a ZRE in the CIITA promoter.

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Repression of CIITA by the Epstein-Barr virus transcription factor Zta is independent of its dimerization and DNA binding

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Abstract (229 words)

Repression of the cellular *CIITA* gene is part of the immune evasion strategy of the γherpes virus Epstein-Barr virus (EBV) during its lytic replication cycle in B-cells. In part this is mediated through down regulation of MHC class II gene expression via the targeted repression of *CIITA*, the cellular master regulator of MHC class II gene expression. The repression is achieved through a reduction in *CIITA* promoter activity initiated by the EBV transcription and replication factor Zta (*BZLF1,* EB1, ZEBRA). Zta is the earliest gene expressed during the lytic replication cycle. Zta interacts with sequence specific elements in promoters, enhancers and the replication origin (ZREs) and also modulates gene expression through interaction with cellular transcription factors and co-activators. Here we explore the requirements for Zta-mediated repression of the *CIITA* promoter. We find that repression by Zta is specific for the *CIITA* promoter and can be achieved in the absence of other EBV genes. Surprisingly, we find that the dimerization region of Zta is not required to mediate repression. This contrasts with an obligate requirement of this region to correctly orientate the DNA contact regions of Zta to mediate activation of gene expression through ZREs. Additional support for the model that Zta represses the *CIITA* promoter without direct DNA binding comes from promoter mapping that shows that repression does not require the presence of a ZRE in the *CIITA* promoter.

Introduction

Epstein–Barr virus infects people and has a life-long association with them, occasionally causing diseases including infectious mononucleosis, Burkitt's lymphoma, Hodgkin's lymphoma and Nasopharygeal carcinoma (Magrath, 2012;

Molyneux *et al.*, 2012; Saha & Robertson, 2011). Epstein–Barr virus infects human B-lymphocytes and epithelial cells and establishes long-term latency in memory B-lymphocytes (Babcock *et al.*, 1998). These cells are largely protected from immune attack by the silencing of viral gene expression. The virus is sporadically reactivated following B-cell activation and differentiation into plasma cells (Crawford & Ando, 1986; Laichalk *et al.*, 2002; Laichalk & Thorley-Lawson, 2005). As EBV enters the lytic replication cycle, it expresses around 90 viral genes that are required for the regulation of viral gene expression, replication of the viral genome, assembly, packaging, and egress of the virion (Farrell, 2005). Many viral genes expressed during viral lytic replication are excellent targets for immune recognition (Adhikary *et al.*, 2006; Long *et al.*, 2011). Attack by the immune system during viral replication would threaten cell survival and thus the successful generation of virions, but EBV has evolved several strategies to evade immune responses during viral lytic replication (Zuo & Rowe, 2012).

An important regulator of EBV lytic replication termed Zta (BZLF1, ZEBRA, EB1) is a transcription factor, a replication factor and it disrupts several signal transduction pathways (Kenney, 2007). Routes by which Zta activates gene expression has been documented for both viral and host promoters. Many promoters are targeted by the interaction of the sequence-specific DNA binding domain of Zta with sequence specific 7-nucleotide DNA elements termed ZREs (for example (Adamson & Kenney, 1999; Bergbauer *et al.*, 2010; Bhende *et al.*, 2004; 2005; Broderick *et al.*, 2009; Dickerson *et al.*, 2009; Flower *et al.*, 2011; Holley-Guthrie *et al.*, 1990; Kalla *et al.*, 2012; Kalla *et al.*, 2010; Karlsson *et al.*,

2008; Kenney *et al.*, 1989; Ramasubramanyan *et al.*, 2012a; Ramasubramanyan *et al.*, 2012b; Sinclair, 2003; Sinclair *et al.*, 1991; Woellmer *et al.*, 2012). At least 32 distinct ZRE sequence variants are specifically recognized by Zta (Flower *et al.*, 2011).

Down regulation of gene expression by Zta has been documented for the TNFR1 gene, through the cellular C/EBP genes (Bristol *et al.*, 2010). Additionally, post-translational modifications of Zta have been shown to reduce the ability of Zta to regulate gene expression, specifically phosphorylation at residue S209 (Asai *et al.*, 2009) and sumoylation through residue K12 (Hagemeier *et al.*, 2010; Murata *et al.*, 2010).

Zta has been shown to down regulate the expression of the master regulator of MHC class II gene expression, *CIITA,* in an EBV-positive B-cell line, with both protein and RNA levels decreasing following induction of EBV lytic cycle activation (Li *et al.*, 2009). The product of *CIITA* is a non-DNA-binding cellular transcriptional co-activator, which acts through interaction with DNA-bound proteins that lack integral activation domains. *CIITA* activates the expression of MHC Class II genes (Chang *et al.*, 2002) and the reduced expression of *CIITA* observed in B-cells undergoing lytic cycle correlates with the reduced expression of MHC class II observed at the cell surface (Li *et al.*, 2009). Repression of *CIITA* gene expression is also driven by the related γherpesvirus KSHV (Cai *et al.*, 2013). Here, we investigate the route by which Zta represses *CIITA* expression.

Results

CIITA promoter is specifically repressed by the EBV Zta protein

The effect of Zta expression on the activity of the *CIITA* promoter and the viral *BHLF1* promoter were compared in EBV-positive Raji cells following co-transfection of reporter constructs with an expression vector for a polyhistidine tagged version of Zta (Bailey *et al.*, 2009). The impact of Zta expression was expressed relative to the maximal activity for each promoter (Fig. 1(a) and (b)). Expression of Zta repressed the *CIITA* promoter, whilst in the same experiment it dramatically activated expression of a viral promoter containing ZREs (*BHLF1)*. This provides confirmation of the results of Li *et al.* showing that a short region of the *CIITA* promoter is sufficient to mediate repression following Zta expression (Li *et al.*, 2009). The repression of *CIITA* promoter activity could result from the overexpression of a transactivator domain that non-specifically sequesters basal transcription factors or co-activators, thereby inhibiting all RNA polymerase II dependent transcription. To address whether this was the case, we undertook experiments to explicitly question whether Zta repressed other promoters. We generated promoter-reporter gene constructs for two viral promoters, *BFLF2* and *BLLF3*. The impact of His-Zta expression on each promoter was assessed in Raji cells (Fig. 1 (c,d)). This showed that neither *BLLF3* nor *BFLF2* promoters were repressed by Zta expression. We further investigated the repression of *CIITA* in BL cells by following two downstream targets of CIITA expression, HLA-DOA and HLA-DBM. Both are down regulated at the RNA level following Zta expression in BL cells (Supplementary Fig. 1).

As Raji cells contain an EBV genome, changes in viral gene expression may occur as a consequence of activating a partial lytic replication cycle through the expression of Zta (Kallin & Klein, 1983). In order to question whether Zta relies on additional viral components to repress *CIITA* expression, we introduced the *CIITA* promoter-reporter gene into an EBV-negative sub-clone of Akata Burkitt's lymphoma cells (AK31) (Jenkins *et al.*, 2000). In this cell background we saw that co-expression of Zta drove repression of the *CIITA* promoter-reporter gene around 5-fold (Fig. 2). This clearly demonstrates that Zta-mediated repression of *CIITA* does not depend on additional EBV genes.

To explore the relevance of post-translational modifications of Zta to the Zta-mediated repression of the *CIITA* promoter, we generated mutants of Zta at amino acid residues K12 and S209 to prevent either sumoylation or phosphorylation. Following transfection we found that neither post-translational modification was required for Zta to repress the *CIITA* promoter (Table 1).

Domains of Zta mediating repression of CIITA promoter

We then explored which domains of Zta protein mediate the repression of *CIITA*. Two versions of Zta were generated; both of these retain the nuclear localization signal (Mikaelian *et al.*, 1993). One mutant omits the N-terminal transactivation domain (ZtaΔTA), this protein was previously shown to be able to bind to DNA but not to transactivate a reporter construct (Packham *et al.*, 1990). The second omits the dimerization and C-terminal region (ZtaH199ter) and has been shown previously to be unable to bind DNA (Hicks *et al.*, 2003) or to form dimers (Schelcher *et al.*, 2005) (Fig 3(a)). Following transfection into Raji cells we find

148 that deletion of the transactivation domain ablates the ability of Zta to repress
149 the *CIITA* promoter, despite the proteins being expressed at an equivalent level
150 (Fig. 3(b)). In contrast, deletion of the dimerization and C-terminal regions of Zta
151 only resulted in a small reduction in the repression of the *CIITA* promoter (Fig.
152 3(c)). The slightly lower level of repression observed with ZtaH199ter might
153 result from the reduced abundance of this form of the protein. Taken together
154 these data show that a major component of Zta mediated repression of the *CIITA*
155 promoter occurs independently of a need for Zta to form dimers.
156
157 It has been shown previously that a Zta binding site within the *CIITA* promoter
158 allows repression by Zta (Li *et al.*, 2009). Our data show that dimerization is not
159 an obligate requirement for repression, implying that DNA binding is not
160 required. To explore this further, we assessed the promoter for potential ZREs
161 (Flower *et al.*, 2011) and found only the one, which was shown to be a Zta
162 binding site previously (Li *et al.*, 2009). We confirm using Chromatin
163 precipitation coupled to next generation DNA sequencing that Zta binds to the
164 promoter region of CIITA (Supplementary Fig. 2), but note that this does not
165 distinguish between direct and indirect binding. To evaluate the relevance of the
166 potential ZRE, we generated a promoter reporter construct in which the region
167 containing a ZRE was deleted (Fig. 4(a)). Both of these promoters are expressed
168 at equivalent levels (Table 2). Both full length Zta and the dimerization-deficient
169 mutant Zta-H199ter repressed the promoter missing the ZRE (Fig. 4(b)). This
170 supports our contention that the ability of Zta to repress the expression of *CIITA*
171 does not rely on direct DNA binding.
172

From these data we devised a model to account for Zta mediated activation and repression of gene expression. In cells expressing MHC class II, CIITA expression is driven by the interaction of cellular factors (RNA polymerase II and cellular co-activators) (Fig. 5(a)). Once Zta is expressed it interferes with the activation machinery operating at the CIITA promoter, without the need to dimerize or bind to the promoter (Fig. 5(b)).

Discussion

The EBV protein Zta is often described as the master regulator of EBV lytic cycle replication. Indeed, the ability of Zta to regulate viral gene expression is crucial to the success of viral lytic replication, as mutation of the *BZLF1* gene in recombinant EBV demonstrates (Feederle *et al.*, 2000). The activation of viral gene expression is considered to occur through the interaction of Zta with sequence specific ZREs in the promoters of viral genes, and the attraction of co-activator proteins such as p300, TFIID and other RNA polymerase II components to the promoters (Lieberman & Berk, 1991; 1994) (Fig. 5 and Supplementary Fig. 3). Recent genome-wide analyses have shown that Zta has extensive interactions across the EBV genome and a specific role in the transcriptional activation of many viral promoters (Bergbauer *et al.*, 2010; Ramasubramanyan *et al.*, 2012a).

Li discovered that Zta-mediated repression of CIITA expression occurs in EBV-positive Raji cells, but did not investigate whether other viral genes are required for the repression (Li *et al.*, 2009). We confirm this and furthermore we show that the expression of two CIITA-dependent genes are also down regulated. As Zta expression in EBV positive BL cells, is sufficient to initiate the viral lytic

198 replication cycle, many downstream changes in gene expression are expected,
199 and it is important to determine whether repression requires Zta action alone or
200 whether it acts in concert with additional viral proteins. Our demonstration that
201 Zta is able to repress the *CIITA* promoter in an EBV-negative BL cell line
202 unequivocally demonstrates that Zta-mediated repression does not require
203 other viral gene products.
204
205 The relevance of two forms of post-translational modification of Zta that have
206 been described as transcriptionally repressive was explored. The involvement of
207 phosphorylation at S209 by the viral protein kinase *BGLF4* (Asai *et al.*, 2009) was
208 investigated using the phospho-mimetic mutant version of Zta S209D and the
209 phosphorylation dead mutant version Zta S209A. Covalent addition of SUMO at
210 K12 (Hagemeier *et al.*, 2010; Murata *et al.*, 2010) was assessed using the non-
211 sumoylatable mutant version Zta K12R. Both of these post-translational
212 modifications have been described as transcriptionally repressive (Asai *et al.*,
213 2009; Hagemeier *et al.*, 2010; Murata *et al.*, 2010). As none of these Zta mutants
214 compromised the ability of Zta to repress the *CIITA* promoter, we conclude that
215 neither post-translational modification is likely to be responsible for the
216 observed repression of the *CIITA* promoter by Zta.
217
218 Zta also regulates gene expression by disrupting transcriptional activation by
219 NFκB and p53 (Morrison & Kenney, 2004; Zhang *et al.*, 1994). This occurs
220 through physical interactions between Zta and the p65 component of NFκB and
221 between Zta and p53 protein (Morrison & Kenney, 2004; Zhang *et al.*, 1994).
222 However, it is unlikely that either NFκB or p53 plays a role in Zta-mediated *CIITA*

repression, as both require the dimerization region of Zta, which is not necessary for repression of *CIITA*. In addition, mutation of the NFκB interaction site in the *CIITA* promoter does not alter either basal expression or Zta mediated repression (NB, AJS unpublished data). It is intriguing that Zta has been shown previously to modulate expression of a viral promoter (Zp) without the need to bind directly to DNA (Flemington *et al.*, 1994).

A previous study suggests that Zta repression of the CIITA promoter is driven through the interaction of Zta with a single ZRE within the promoter (Li *et al.*, 2009). This is supported by the impact of mutations of the ZRE within the promoter and by the inability of Zta to repress the *CIITA* promoter when the basic region is lost. This study places emphasis on a need of Zta to bind directly to DNA to effect repression. Our experiments support a different conclusion in which Zta represses CIITA expression without binding directly to DNA. We rationalize the need for the basic region of the Zta protein based on a requirement for the nuclear localization domain, which is contained therein (Mikaelian *et al.*, 1993). Without entry to the nucleus, Zta would not be able to repress the CIITA promoter through either direct or indirect DNA binding.

In summary, we show that Zta mediated repression of the *CIITA* promoter can occur without Zta contacting DNA directly, this is supported by the retention of repression when (i) the ZRE is deleted and (ii) by a version of Zta that is defective for dimerization and therefore defective for DNA-binding. This discovery leads us to propose a mechanism to describe gene repression by Zta. In this model the amino terminal region of Zta is able to impede the function of

an essential component of the transcriptionally active CIITA promoter, for example a DNA bound transcription factor or a transcription factor-associated co-activator, thereby preventing its productive association with RNA Pol II and its accessory proteins (Fig. 5).

Materials and Methods

Plasmid constructs.

The *CIITA* promoter (-286 to +54) was cloned with a Kpn I restriction enzyme site included at the 5' end and a Hind III site at the 3' end of the sequence. The promoter was sub-cloned into the pGL3 enhancer plasmid, which contains a luciferase reporter construct down-stream from a multi-cloning site and which includes a distal SV40 enhancer (*Promega).* A 5' deletion version of the promoter was generated (-214 to +54); the location of the 5' end of this promoter is immediately 3' from the ZRE.

The *BHLF1, BFLF2 and BLLF3* promoters were cloned with a BamHI restriction enzyme sites added at the 5' end and Hind III sites at the 3' end. The DNA sequence between co-ordinates 40472 and 40818, 45793 and 44746 and 76186 and 77231 of the EBV genome (Human herpesvirus 4 complete wild type genome Accession: NC_007605.1) were synthesized for the promoter regions for the *BHLF1, BFLF2* and *BLLF3* genes respectively. The promoters were sub-cloned into the pCpGL plasmid (Klug & Rehli, 2006), which is based on pGL3 basic and contains a luciferase reporter construct down stream from a multi-cloning site.

A plasmid driving the expression of hexa-histidine tagged Zta (His-Zta) (Bailey *et al.*, 2009) was used to express His-Zta, compared to the vector control pcDNA3 (*Invitrogen).*

Expression vectors for His-Zta K12R, His-Zta S209A and His-Zta S209D were generated by site directed mutagenesis of His-Zta using the primers shown in Table 3. An expression vector for His Zta-199ter which introduces a termination codon at the amino acid 199 of the Zta coding sequence and His Zta-ΔTA which deletes amino acids 1-133 of Zta were generated by gene synthesis (*Invitrogen*).

Cell culture

Plasmids were introduced into EBV-positive Raji cells (Pulvertaft, 1965) or EBV-negative Akata cells (Jenkins *et al.*, 2000) by electroporation. 1 x 10^7 cells in 0.25ml of medium were incubated with 10μg of plasmid DNA and pulsed with 250V at a capacitance of 975μF in a Gene Pulser II electroporator *(Bio-Rad).*

Luciferase assays

48 hours post-transfection cells were harvested into 250μl of Passive Lysis Buffer (*Promega*) and incubated at room temperature for 15 minutes. The lysed cells were then centrifuged for 10min at 8 krpm and the supernatant was used to determine luciferase activity. 10μl aliquots of each lysate sample were pipetted into a 96-well white luminescence plate and analyzed using luciferase detection kit reagents with a Glomax detection system (*Promega*). A protein concentration assay was undertaken (*Biorad)* and promoter activity was expressed as

luciferase RLU/μg protein. Significance of different promoter activity was assessed using a Student's paired T-test with 2 tail distribution.

Protein analysis

Proteins were extracted from cells by boiling in 2X Laemeli sample buffer and fractionated on Novex protein gels (*Invitrogen).* Following transfer to nitrocellulose membranes the blots were incubated with the Zta specific antibody sc-17503 (*Santa Cruz*) which recognizes the amino-terminal region of Zta, or BZ1 which recognizes the basic and dimerization regions of Zta (Young *et al.*, 1991) or a rabbit polyclonal beta actin antibody (Sigma), followed by detection with HRP-linked secondary antibodies and ECL (Ramasubramanyan *et al.*, 2012b).

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Figure legends

Figure 1. Repression of *CIITA* promoter by Zta is specific.

The *CIITA* (-286 to +54) (a), *BHLF1* (b), *BFLF2 (c)* and *BLLF3* (d) promoter-luciferase plasmids and the indicated expression vectors were introduced cells by electroporation, 48 hours later cells were harvested and the luciferase activity determined. a-d. Promoter activity in Raji cells relative to the maximal activity of *CIITA* promoter (transfected with control plasmid) with the standard deviation from six assays (three replicate samples from each of two separate experiments). For comparisons +/- Zta ** represents p of significant difference <0.01; * represents p of significant difference <0.05. The expression of His-Zta and endogenous protein were analyzed by western blot of proteins from the transfected cells.

Figure 2. Repression of *CIITA* promoter by Zta is independent of other viral proteins.

The *CIITA* (-286 to +54) promoter-luciferase plasmid and the indicated expression vectors were introduced into an EBV-negative sub-clone of the Akata BL cells (AK31) by electroporation, 48 hours later cells were harvested and the luciferase activity determined. Promoter activity is expressed relative to the maximal activity of *CIITA* promoter (transfected with control plasmid) with the standard deviation from six assays (three replicate samples from each of two separate experiments). For comparisons +/- Zta ** represents p of significant difference <0.01;. The expression of His-Zta and endogenous protein were analyzed by western blot of proteins from the transfected cells.

Figure 3. Zta repression of CIITA promoter requires the transactivation domain.

The CIITA promoter-luciferase plasmids (-286 to +54) and either control, His-Zta or His-Zta mutant expression vectors were introduced into Raji BL cells by electroporation. 48 hours later cells were harvested and the luciferase activity and protein concentrations determined. For comparisons +/- Zta ** represents p of significant difference <0.01; * represents p of significant difference <0.05.

a. Schematic of the Zta protein and the two mutant versions that were evaluated. TA is transactivation domain; B is basic DNA contact region; ZIP is dimerization bZIP domain; CT is the Carboxy terminal region (required for dimerisation and replication).

b. Promoter activity of -286 to +54 promoter with His-ZtaΔTA with the standard deviation from six assays (three replicate samples from each of two separate experiments), together with a western blot.

c. Promoter activity of -286 to +54 promoter with His-Zta199ter with the standard deviation from six assays (three replicate samples from each of two separate experiments), together with a western blot.

Figure 4. Zta repression of *CIITA* promoter occurs without binding to the ZRE.

The *CIITA* promoter-luciferase plasmids (either -286 to +54 or -214 to +54) and either control, His-Zta or His-Zta mutant expression vectors were introduced into Raji BL cells by electroporation. 48 hours later cells were harvested and the luciferase activity and protein concentrations determined. For comparisons +/- Zta ** represents p of significant difference <0.01; * represents p of significant difference <0.05. For both His-Zta and His-ZtaH199ter, the significance is equal for each of the different promoters.

a. Schematic of the *CIITA* mutant promoters used in these experiments. The location of the ZRE is indicated by a filled box.

b. The *CIITA* basal promoter activity is shown (open) together with the His-Zta mediated activity (black), with the standard deviation from six assays (three replicate samples from each of two separate experiments). Western blot analysis of protein expression in the transfected cells.

Figure 5. Proposed model to explain Zta mediated gene repression of the *CIITA* promoter.

a. The active *CIITA* promoter is shown. Basal transcription factors are represented as white spheres and co-activators by the stippled oval. RNA polymerase II is represented by the black cloud with transcription indicated by an arrow.

552 b. The ability of a non-DNA binding form of Zta (filled oval) to repress expression
553 of the *CIITA* promoter suggests that some repression can occur without direct
554 DNA contact. The simplest model to account for this has the N-terminal part of
555 Zta blocking the interaction of the basal transcription machinery.
556
557 **Tables**
558 Table 1. Impact of mutation of K12 and S209 on Zta mediated repression

	His-Zta	His-Zta K12R	His-Zta S209A	His-Zta S209D
CIITA promoter activity	1.00	1.00	1.00	1.00
Relative promoter activity following his-Zta expression	0.16	0.07	0.07	0.04
Standard deviation	0.15	0.08	0.01	0.00

559
560 Table 2. Impact of Zta expression on -286 and -214 *CIITA* promoters

	Average luciferase units *CIITA* (-286/+54)	Average luciferase units *CIITA* (-214/+54)
control	440050 +/- 7113	472832 +/- 15031
His-Zta	18448 +/- 464	66785 +/- 1917

561
562 Table 3. Oligonucleotides used to generate mutations.

His-Zta S209A Amino acid serine 209 mutated to Alanine	GGCTGCTGCCAAATCAGCTGAAAATGACAGGCTGC GCC; GGCGCAGCCTGTCATTTTCAGCTGATTTGGCAGCA GCC
His-Zta S209D Amino acid serine 209 mutated to Glutamic acid	GGCTGCTGCCAAATCAGATGAAAATGACAGGCTGC GCC; GGCGCAGCCTGTCATTTTCATCTGATTTGGCAGCA GCC
His-Zta K12R Amino acid lysine 12 mutated to arginine	CTCGACTTCTGAAGATGTAAGATTTACACCTGACC CATACC; GGTATGGGTCAGGTGTAAATCTTACATCTTCAGAA GTCGAG

Figure 1

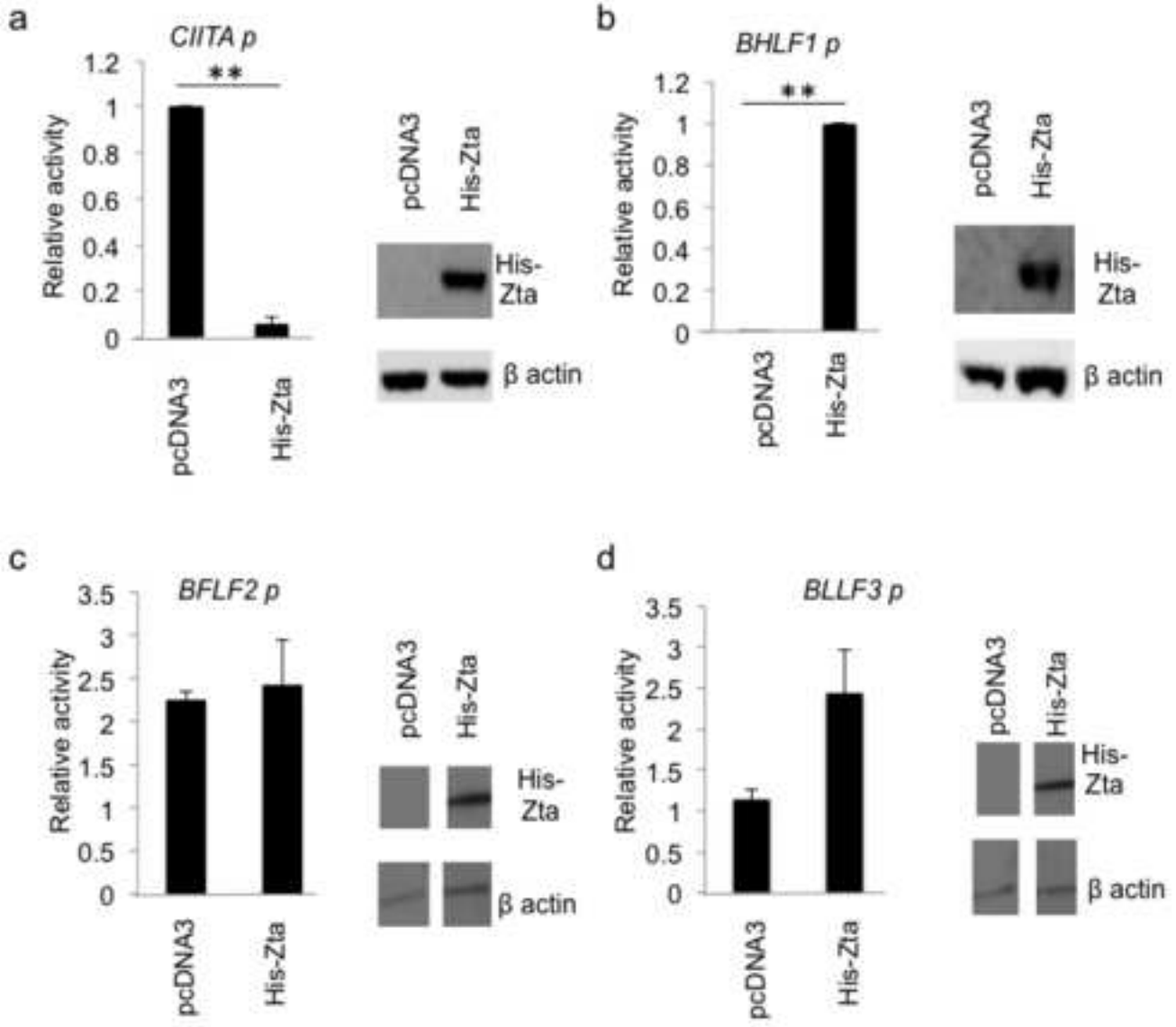


Figure 2

Click here to download Figure fig2.tiff

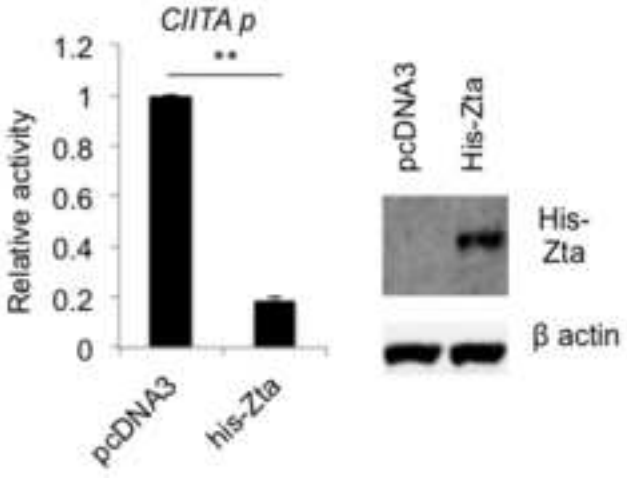


Figure 3

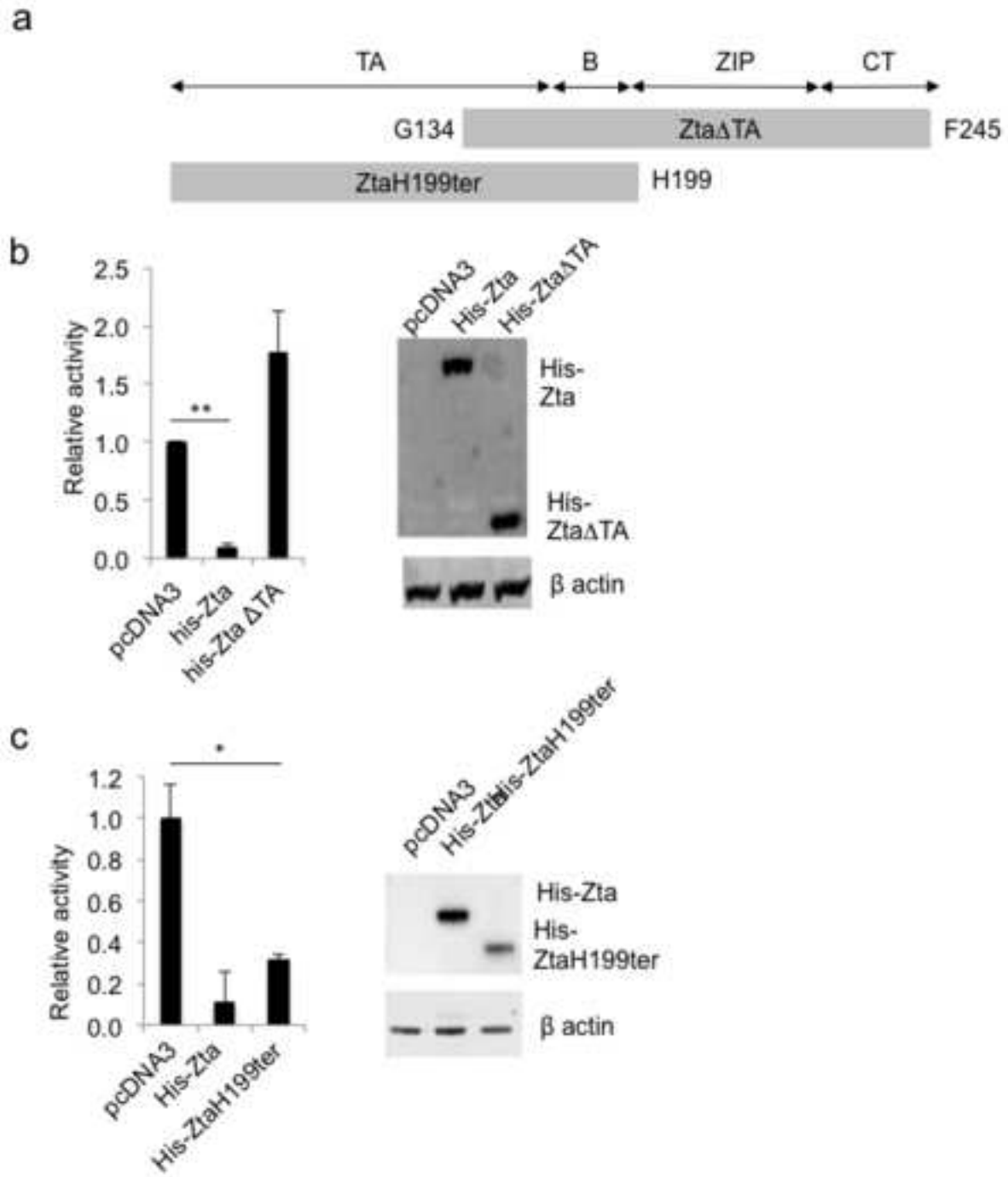


Figure 4

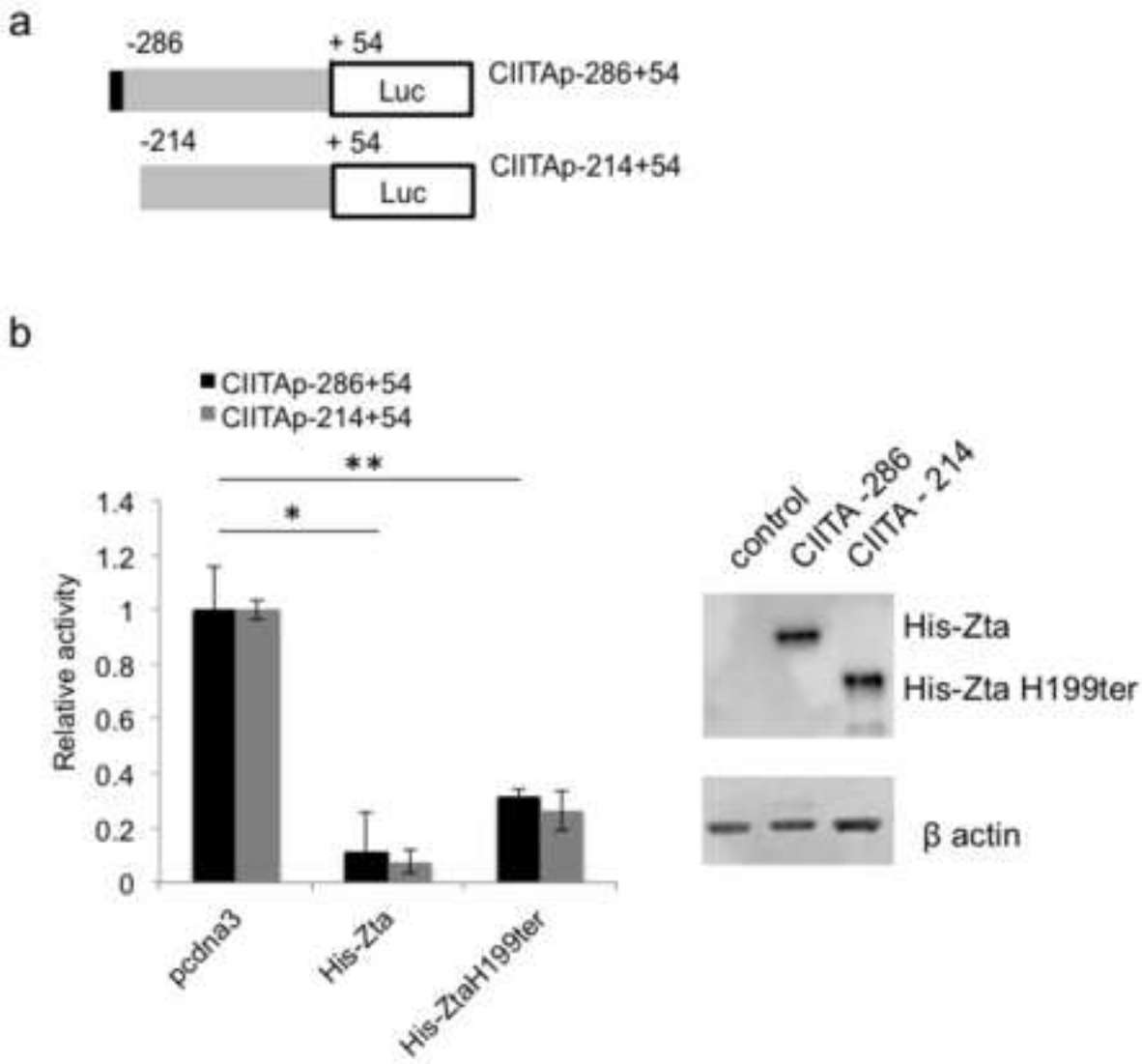
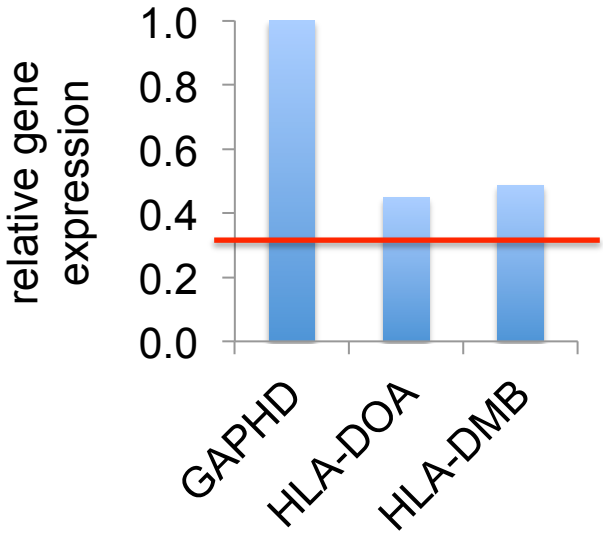
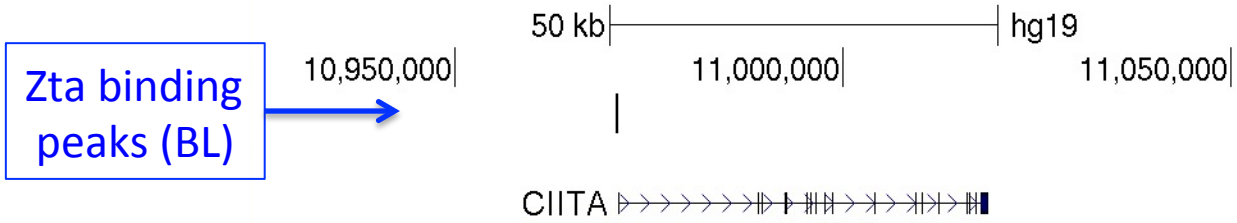


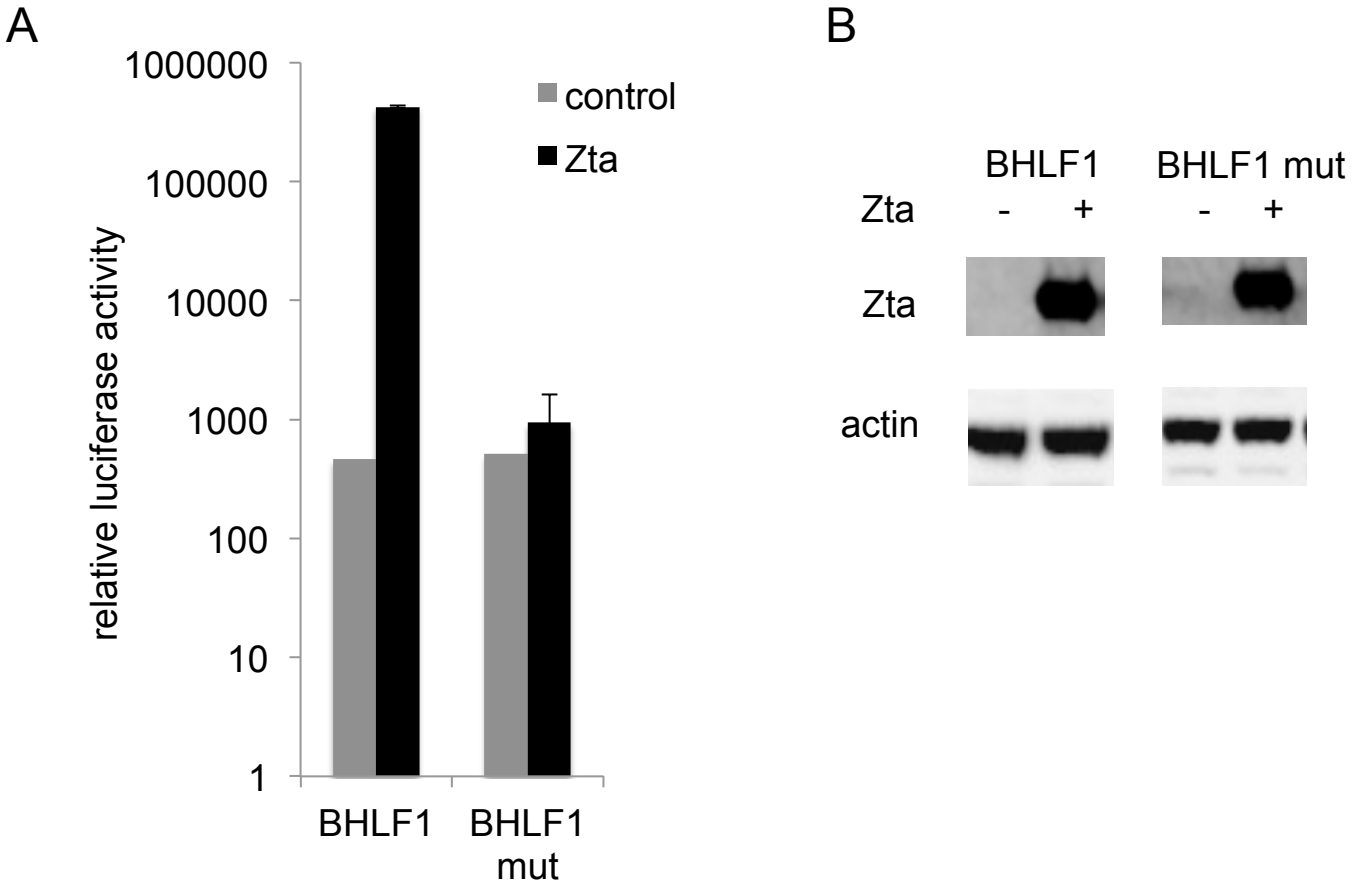
Figure 5



Supp Figure 1. CIITA downstream targets.
The expression of targets of CIITA were investigated by RNA seq as described in Ramasubramanyan et al 2015).
The relative change in expression following Zta expression in Akata BL cells was determined and presented (FDR<0.05).
The red line indicates the maximum reduction expected as only 70% of cells express Zta.



Supp figure 2. Interaction of Zta with the CIITA pomoter in BL cells.
The interaction of Zta with the CIITA locus was undertaken by ChIP-seq (as described in Ramasubramanyan et al 2015). The locus is shown chr16:10,923,269-11,066,626. A peak of binding is seen as a bar spanning the TSS of the gene in Akata BL cells undergoing lytic replication cycle.



Supp Figure 3. Activation of BHLF1 promoter depends on ZREs in BL cells.
The viral *BHLF1* promoter-luciferase plasmid and a version that has a mutation of each ZRE within the promoter was introduced into BL cells by electorporation together with a his-Zta expression vector or control plasmid. 48 hours later cells were harvested and the luciferase activity and protein concentrations determined.

A. Relative activity of promoters (log10 scale).
B. Western blot showing his-Zta and actin expression.